THE EFFECTS OF HYPOPHYSECTOMY AND BOVINE PROLACTIN ON SALT FLUXES IN FRESH-WATER-ADAPTED FUNDULUS HETEROCLITUS

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Some of the characteristics of salt balance in the euryhaline killifish *Fundulus heteroclitus* have been outlined by us elsewhere (Potts and Evans, 1966). One of the major features of osmotic regulation in this fish is a marked reduction in the sodium and chloride fluxes on adaptation to fresh water. A similar reduction has been observed in several euryhaline teleosts including the stickleback *Gasterosteus aculeatus* (Mullins, 1950), the rainbow trout *Salmo gairdneri* (Gordon, 1962), *Fundulus kansae* (Fleming and Kamemoto, 1963), a blenny *Blennius pholis* (House, 1963) and the flounder *Platichthys flessus* (Motais and Maetz, 1964). Measurements of the drinking rate in *Fundulus heteroclitus* (Potts and Evans, 1966) and in *Platichthys flessus* (Motais and Maetz, 1964) show that the larger part of the influx in sea water takes place through the body surface, not through the gut. The site of this influx is uncertain but may well be the gills. Whatever the site it is clear that the body surface of sea-water-adapted fish is relatively permeable to ions but the low fluxes found in fresh-water-adapted fish show that they are relatively impermeable to ions.

Burden (1956) first demonstrated that hypophysectomized killifish were unable to survive in fresh water although they could survive in saline solutions. Later Pickford and Phillips (1959) showed that ovine prolactin promoted survival of hypophysectomized killifish in fresh water. Similar results have been obtained with several other genera including *Mollienesia* (Mollies) (Ball, 1962), *Poecilia* (Ball and Olivereau, 1964), *Xiphophorus* (platyfish and sword-tails) (Schreibman and Kallman, 1962) and *Tilapia* (Handin, Nandi and Bern, 1964). In addition Schreibman and Kallman (1962) and Ball *et al.* (1965) have demonstrated that pituitary transplants enable hypophysectomized fish to survive indefinitely in fresh water. Schreibman and Kallman (1966) have recently reviewed the problem of hypophysectomy and survival in fresh-water fishes.

In the light of this evidence of the influence of prolactin on the osmoregulatory ability of teleosts the effects of hypophysectomy and prolactin on the salt fluxes of *Fundulus* have been examined.

Materials and Methods

The fish used were *Fundulus heteroclitus*, weighing between 2 and 5 gm., collected by the Supply Department of the Woods Hole Marine Biological Laboratory. Sea-water-adapted fish were kept in running sea water which varied slightly in salinity during the course of the work, from between 420 to 435 m*M* Na/L., 30.7–31.8‰ salinity, and between 19° and 21° C. in temperature. Fish adapted to 40% sea water, approximately isosmotic with the blood, and to fresh water were kept in aerated plastic tanks which stood in trays of running sea water so that the temperature of the tanks was approximately that of the sea water. Artificial fresh water was prepared by diluting sea water to 1 m*M* Na/L. with distilled water. The fish were fed every two or three days with chopped clam.

Sodium fluxes were measured by the methods described elsewhere (Potts and Evans, 1966). The fish were loaded by placing them in a solution containing ^{24}Na for two hours. The fish were then washed and the active solution replaced by an inactive solution. The efflux was measured after two hours in the inactive solution. All handling was kept to a minimum. The influx is obtained in absolute terms, *i.e.,* the amount of sodium which has entered the fish/hr., but the efflux is obtained in terms of the rate constant, *i.e.,* that fraction of the activity which leaves/unit time.

Hypophysectomy was performed by a method similar to that of Abramowitz (1937) and Handin *et al.* (1964). The fish were anaesthetized by 1:5000 MS 222. The parasphenoid was cut by iridectomy scissors and the pituitary removed by gentle suction. The animals were allowed to recover in 40% sea water and were used for experiment after normal feeding had been resumed, usually after one to two weeks. Bovine prolactin (NIH-P-BI, 13 International units/mg.) was provided by the NIH Pituitary Hormone Distribution Program. Prolactin was injected intraperitoneally in isotonic NaCl containing 168 m*M* NaCl/L., and 500 mg. prolactin/L.

Unless otherwise stated fish were injected with 20 γ prolactin/fish (*ca.* 5 γ/gm. fish) 48 hours before the experiment and injected again with 20 γ/fish 24 hours before the experiment. After the second injection the fish were transferred to fresh water. Each injection was equal to about 1% of the weight of the fish. This is equivalent to only half an hour of normal drinking in 40% sea water (Potts and Evans, 1966) or to one hour of urine production in fresh water (Stanley and Fleming, 1964). The injections are unlikely to alter water balance significantly 24 hours later.

Results

After recovery from hypophysectomy the fish survived for at least several weeks in 40% sea water but after transfer to fresh water the fish became aesthenic in a few hours and the majority died within 24 hours. Burden found that hypophysectomized *Fundulus* would survive for several days in fresh water at 15° C. The more rapid deterioration of our fish may have been due to the higher temperature. Control fish survived indefinitely in the fresh water.

Sodium and bromide fluxes in hypophysectomized Fundulus

The rate constants of sodium and bromide efflux from hypophysectomized *Fundulus* in the two hours following transfer from 40% sea water to fresh water are shown in Table I. Salt loss is clearly much higher in hypophysectomized fish.

The rate of influx of sodium into hypophysectomized fish in fresh water was similar to that into normal fish. The mean rate of influx into a small series of four hypophysectomized fish in the two hours following transfer from 40% sea water was 0.50 μM/gm./hr. (range 0.63–0.31). In normal fish fully adapted to fresh water the influx averages 0.58 ± 0.10 μM/gm./hr. (N = 16) (Potts and Evans, 1966). It is clear that the hypophysectomized fish is at a disadvantage in fresh water. As the sodium content of the fish will decline during the experiment the rate of loss will vary with time. However, if the initial sodium content of the fish were 60 μM Na/gm. (Potts and Evans, 1966) and the efflux rate constant were invariable, the initial rate of loss would be about 12 μM/gm./hr. while the influx would be only 0.5–0.6 μM Na/gm./hr.

In normal fish adapted to fresh water the greater part of the sodium loss takes place through the body surface (*ca.* 0.36 μM/gm./hr.) while the remainder (*ca.* 0.22 μM/gm./hr.) is lost through the kidney and gut (Potts and Evans, 1966; Meier and Fleming, 1962; Stanley and Fleming, 1965). In an attempt to isolate the site of the increased loss of salt, hypophysectomized fish were transferred to fresh water four hours after the anus and excretory opening had been ligated. The mean rate constant of the sodium efflux during the two-hour period after transfer was then 0.208 ± 0.039 (N = 9). Hence the greater part of the loss probably takes place through the body surface.

TABLE I

Rate of constants of sodium and bromide efflux from normal and hypophysectomized Fundulus in various media; h^{-1} 20°C.

	Sea water	40% sea water	Fresh water (during 2 hours following transfer from 40% sea water)	Fresh water (adapted fish)
Sodium				
Hypophysectomized fish	0.446 + 0.041 (7)	0.209 ± 0.07 (4)	0.192 ± 0.080 (11)	———
Normal fish	0.462 ± 0.024 (19)	0.175 ± 0.027 (14)	0.050 ± 0.013 (6)	0.0114 ± 0.003 (8)
Bromide				
Hypophysectomized fish	———	———	0.255 ± 0.032 (17)	———
Normal fish	0.492 ± 0.027 (9)	0.087 ± 0.007 (27)	0.134 ± 0.023 (18)	0.037 ± 0.004 (14)
			Mean ± S.E.	(No. of dets.)

The effect of prolactin

Hypophysectomized fish which received 20 γ of prolactin every two days survived well in fresh water. The mortality that occurred, *ca.* 10%/week, may be attributed to the handling associated with the injections. The mean rate constant of 6 fish maintained in fresh water for 24 hours after receiving the second 20 γ injection of prolactin was 0.026 ± 0.006. Three hypophysectomized controls which had received no prolactin were all dead after 24 hours. In comparison normal fish, fully adapted to fresh water, have an efflux constant of 0.0114 ± 0.003 (N = 8) (Potts and Evans, 1966).

Normal fish adapted to fresh water contain about 50 μM Na/gm. If the hypophysectomized fish receiving prolactin contained the same quantity an efflux rate constant of 0.026 h^{-1} would correspond to a sodium loss of 1.3 μM/gm./hr. The mean measured influx into the four hypophysectomized fishes receiving prolactin was 1.0 μM Na/gm./hr. As the fish survived almost indefinitely, a balance must be struck between efflux and influx. How this is achieved requires further investigation. If the efflux is initially greater than the influx the blood concentration will decline. This may stimulate the uptake system above its normal levels. Alternatively as the total body sodium declines the absolute loss will decline in proportion even if the rate constant does not change.

Discussion

The ability of prolactin to prolong the survival of hypophysectomized fish in fresh water has been known for seven years but the physiological basis of this action has not been clear. *A priori* two simple explanations present themselves. Prolactin might facilitate salt uptake in fresh water and/or it might reduce salt loss. In the latter case the loss might be reduced either at the body surface and/or in the kidney. The experiments reported above show that prolactin acts primarily in reducing salt loss. In the absence of prolactin the blood concentration will fall as loss exceeds uptake. Pickford, Pang and Sawyer (1966) have shown that when hypophysectomized *Fundulus heteroclitus* fails in fresh water the blood concentration lies in the region of 0.25–0.29 M/L. compared with a normal concentration of 0.37 M/L. The effect of hypophysectomy on salt uptake requires further investigation. The survival of hypophysectomized fish for more than a few hours suggests that some compensation for the large losses has taken place. Any decline in blood concentration might be expected to stimulate salt uptake to some extent, but any such effect will be secondary.

The ligation experiments suggest that the greater part of the salt loss for hypophysectomized fish occurs at the body surface. The difference observed between ligated and non-ligated hypophysectomized fish, 0.208 ± 0.039 and 0.235 ± 0.089, respectively, is not significant. Stanley and Fleming (1960) have shown that prolactin does affect renal function. In the plains killifish, *F. kansae,* prolactin was found to increase urine flow but to reduce urine sodium concentration in fish adapted to fresh water. It is not clear whether or not the overall renal sodium loss was increased or decreased.

The relationship between the effects of prolactin in these experiments and the normal function of the pituitary in the control of osmoregulation must now be considered. It has been shown that adaptation to fresh water by euryhaline teleosts is associated with a marked reduction in the extrarenal loss of sodium (Motais and Maetz, 1964; Potts and Evans, 1966). This is brought about in *Fundulus* by a reduction of the permeability of the body surface to ions (Potts and Evans) although in other species exchange diffusion plays an important part in the apparent reduction of sodium loss (Maetz, personal communication). In *Fundulus* a significant reduction in permeability may be induced by the immersion of sea-water-adapted fish in fresh water for only 10 minutes. Once induced the reduced permeability is maintained for several hours. It is tempting to suggest that this reduction of permeability is brought about by the release of a prolactin-like hormone from the fish pituitary. It is significant in this respect that hypophysectomized fish survived well in sea water and the rate constants of sodium exchange were similar to those in normal fish (Table I). Similarly the rate constant of hypophysectomized fish in fresh water, and hence their permeability, was similar to that of fish adapted to 40% sea water.

A prolactin-like hormone has been identified in *Fundulus* and in the fresh-water carp but not in the marine cod (*Cadus*) or hake (*Urophycis*) (Grant and Pickford, 1959). This teleost prolactin-like hormone resembles mammalian prolactin but is not identical with it. The teleost prolactin-like hormone possesses red eft water drive activity (Grant and Pickford, 1959) but lacks pigeon crop sac stimulating activity (Nicoll and Bern, 1964). Similarly frog prolactin shows some but not all the characteristics of mammalian prolactin (Chadwick, 1966).

A prolactin-like hormone is not essential for the survival of all teleosts in fresh water. Both the eel *Anguilla anguilla* and the golfish *Carassius auratus* will survive in fresh water following hypophysectomy (Fontaine, Callamand and Olivereau, 1949; Schreibman and Kallman, 1966), although the eel at least also produces a prolactin-like hormone (Ball and Olivereau, 1964). In the case of the eel survival may be due to its very low permeability. Normal silver eels will survive "readily" in glass-distilled water for three months and the rate of sodium loss in fresh water is of the order of 11 μM/kg./hr. compared with 580 μM/kg./hr. in *Fundulus* (Chester Jones, Henderson and Butler, 1965). At low temperatures even hypophysectomized *Fundulus* will survive in fresh water for several days (Burden, 1956) so it must be very close to salt balance in these conditions.

Attempts by the authors to induce a state of low permeability by the injection of prolactin into sea-water-adapted *Fundulus,* comparable with that induced by a short treatment with fresh water, were not successful. This may indicate that further stimuli are required to bring about a state of low permeability in addition to prolactin alone. On the other hand more success might be obtained with ovine prolactin or better still with a fish prolactin-like hormone. Ovine prolactin supports hypophysectomized *Fundulus* rather better than bovine (Pickford, Robertson and Sawyer, 1965).

Pickford, Pang and Sawyer (1966) have recently suggested that prolactin prolongs survival in fresh water by its action on the fish mucous cells. The greater part of the extrarenal sodium exchange probably takes place through the gills rather than through the general body surface. Mucus might reduce loss over the gills by increasing the thickness of the non-stirred layer but a direct action on the gill epithelium is also possible.

Olivereau (1966) found that prolactin had a thyroid-stimulating action in hypophysectomized eels (*Anguilla*) but thyrotropin does not prolong survival in hypophysectomized platyfish (*Xiphophorus maculatus*) in fresh water (Schreibman and Kallman, 1966).

There is some evidence that hypophysectomy reduces urine flow in fresh water while prolactin increases it again. Chester Jones *et al.* (1965) found that urine flow in hypophysectomized eels was less than half normal while Stanley and Fleming (1965) found that hypophysectomized *F. kansae* had low rates of urine production which were increased by prolactin. Their results may imply that prolactin increases water permeability but a low permeability to water would not be a disadvantage in fresh water.

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Summary

Hypophysectomized *Fundulus* in fresh water lost sodium several times as rapidly as normal fish. The greater part of the loss takes place extrarenally. Prolactin reduces the loss almost to normal levels, thus prolonging survival. The possible functions of prolactin in osmoregulation in normal fish are discussed.

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